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## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/US93/02389 <b>(22) International Filing Date:</b> 17 March 1993 (17.03.93) <b>(30) Priority data:</b> 07/852,511 17 March 1992 (17.03.92) US <b>(71) Applicant:</b> CHILDREN'S HOSPITAL MEDICAL CENTER OF NORTHERN CALIFORNIA [US/US]; 747 Fifty Second Street, Oakland, CA 94609 (US). <b>(72) Inventor:</b> PERRINE, Susan, P. ; 2682 Longview Drive, Richmond, CA 94806 (US). <b>(74) Agents:</b> SCHLICHER, John, W. et al.; Townsend and Townsend Kourie and Crew, Steuart Street Tower, 20th Floor, One Market Plaza, San Francisco, CA 94105 (US).		<b>(81) Designated States:</b> AT, AU, BB, BG, BR, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, LK, LU, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG).  <b>Published</b> <i>With international search report.</i>
<b>(54) Title:</b> PHARMACEUTICAL COMPOSITIONS AND METHODS USING ISOBUTYRAMIDE FOR TREATING BETAGLOBIN DISORDERS  <b>(57) Abstract</b>  This invention relates to treatment of betaglobin disorders, such as sickle-cell anemia and beta-thalassemia, by administering compositions of isobutyramide.		

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PHARMACEUTICAL COMPOSITIONS AND METHODS  
5        USING ISOBUTYRAMIDE FOR TREATING BETAGLOBIN DISORDERS

STATEMENT AS TO RIGHTS TO INVENTIONS MADE  
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10        The U.S. Government has a paid-up license in this invention and the right in limited circumstances to require the patent owner to license others on reasonable terms as provided for by the terms of grant no. 37118 awarded by the Heart, Lung, and Blood Institute, Department of Health and Human Services.

15        BACKGROUND OF THE INVENTION

20        Betaglobin is a polypeptide subunit of hemoglobin A, the principal hemoglobin in adult humans. Hemoglobin A is the principal oxygen carrier in blood. Hemoglobin A is made up of four subunits, two alphaglobin chains and two betaglobin chains. Several diseases are characterized by the production of abnormal betaglobin and impaired hemoglobin S, as in sickle-cell anemia, or from the production of no or deficient amounts of betaglobin and hemoglobin A, as in beta-  
25        thalassemia. These diseases have long been recognized to arise from genetic defects, such as a single mutation in the betaglobin gene in sickle-cell anemia.

30        There is no pharmaceutical composition or method in use for the effective treatment of betaglobin disorders.

SUMMARY OF THE INVENTION

35        This invention is directed to pharmaceutical compositions and methods employing isobutyramide for treating disorders arising from betaglobin deficiencies. This invention relates to use of isobutyramide to treat these disorders by stimulating production of fetal hemoglobin, hemoglobin F.

## DESCRIPTION OF THE INVENTION

The invention provides pharmaceutical compositions containing isobutyramide and methods for treating betaglobin disorders by administering isobutyramide.

5           Isobutyramide is a chemical having the formula  $(\text{CH}_3)_2\text{CHCONH}_2$ . Isobutyramide may be produced by routine synthesis procedures and is available from chemical suppliers, such as Aldrich Chemical Co.

10           The pharmaceutical compositions of this invention contain isobutyramide. These compositions are useful in treatment of disorders involving the failure to produce normal betaglobin entirely or in sufficient amounts. The production of abnormal betaglobin or the production of betaglobin in insufficient amounts leads to the production of abnormal  
15   hemoglobin, such as hemoglobin S, or deficient amounts of hemoglobin A. These disorders are generally referred to as beta-hemoglobinopathies and beta-thalassemias. They are referred to here simply as betaglobin disorders. The most common disorders of this type are sickle-cell anemia and beta-  
20   thalassemia. Examples of other betaglobin disorders are hemoglobin C, hemoglobin E, hemoglobin Lepore, hemoglobin SC, hemoglobin S-beta 0 or + thalassemia, hemoglobin E-beta-thalassemia, delta beta-thalassemia, and other combinations of these disorders.

25           The compositions and methods of this invention are useful for treatment of the betaglobin disorders in mammals, including humans. These compositions and methods stimulate the production of fetal hemoglobin, also referred to as hemoglobin F, to a level that achieves therapeutic treatment  
30   of these disorders. Therapeutic treatment of these disorders may involve administration to patients prophylactically, that is to prevent, retard, or reduce the severity of future occurrence of the disease or its clinical manifestations. The ability of isobutyramide to stimulate production of fetal  
35   hemoglobin permits a new practical therapy of these conditions.

          The compositions of the invention contain a therapeutically effective amount of isobutyramide with a phar-

maceutically acceptable carrier. A pharmaceutically acceptable carrier may be any compatible, non-toxic substance suitable to deliver the isobutyramide to the patient. The compositions may contain other pharmaceutically acceptable substances as required to approximate physiological conditions such as pH adjusting and buffering agents, dispersing agents, toxicity adjusting agents, flavoring agents and the like. The concentration of isobutyramide in these formulations may vary and will be selected primarily based on the particular dosage and mode of administration selected. Methods for preparing administrable compositions are well known to those skilled in the art. The pharmaceutical compositions of isobutyramide are useful for oral or parenteral administration. The compositions may be formulated in a variety of dosage forms, such as tablets, capsules, oral solutions or suspensions, sterile parenteral solutions or suspensions, transcutaneous patches, slow-release implants, and indwelling implant devices.

Preferably, the pharmaceutical compositions are administered orally. For oral administration, solid or fluid dosage forms can be prepared. For preparing solid compositions such as tablets, the isobutyramide is mixed with conventional ingredients, such as talc, magnesium stearate, and functionally similar materials, as pharmaceutical carriers. Capsules are prepared by mixing the isobutyramide with an inert pharmaceutical diluent and filling the mixture into a hard gelatin capsule. Soft gelatin capsules are prepared by machine encapsulation of a slurry of the isobutyramide with an acceptable vegetable oil, light liquid petrolatum or other inert oil. Fluid unit dosage forms for oral administration such as syrups and suspensions can be prepared. The isobutyramide can be dissolved in an aqueous vehicle together with sugar, sweetening and flavoring agents and preservatives to form a syrup. Suspensions can be prepared with an aqueous vehicle and a dispersing agent such as acacia, tragacanth, methylcellulose and the like.

This invention also provides compositions for parenteral administration, i.e. subcutaneous, intramuscular,

intravenous, or transcutaneous administration. For parenteral administration, fluid dosage forms are prepared utilizing the isobutyramide and a sterile carrier, water being preferred. The isobutyramide, depending on the carrier and concentration used, may be either suspended or dissolved in the carrier. In preparing solutions, the isobutyramide can be dissolved in water and sterilized before filling into a suitable vial or ampule. Adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the carrier. The compositions can be frozen after filling into the vial and the water removed under vacuum. The dry lyophilized powder is then sealed in the vial and an accompanying vial of water for injection is supplied to reconstitute the liquid prior to use. Parenteral suspensions can be prepared in substantially the same manner except that the compositions are suspended in the carrier instead of being dissolved. A surfactant or wetting agent is included in the compositions to facilitate uniform distribution of the isobutyramide.

The compositions containing isobutyramide may be administered for the therapeutic treatment of betaglobin disorders, including prophylactic treatment. In treatment of patients diagnosed with a betaglobin disorder, compositions are administered to a patient in an amount sufficient to produce fetal hemoglobin at levels that reduce the severity of the betaglobin disorder or its clinical manifestations. In prophylactic treatment, the compositions may be administered to a patient who may be at risk of having a betaglobin disorder, but has not been diagnosed as having such a disorder. In prophylactic therapy, compositions are administered to such a patient in an amount sufficient to produce fetal hemoglobin at levels that prevent, retard or reduce the severity of the betaglobin disorder or its clinical manifestations. An amount adequate to accomplish any of these effects is referred to as a "therapeutically effective" amount. Unit dosages effective for this use will depend upon the severity of the disorder and the general state of the patient's health, but generally will range from about 5 to about 500 mg of isobutyramide per kilogram of body weight,

with dosages of from 10 to 250 mg per kilogram being commonly preferred, and dosages of about 15 to 200 mg per kilogram more preferred. The compositions are administered in multiple unit dosages over a period of time with a frequency and duration sufficient to yield a "therapeutically effective" amount, i.e., an amount sufficient to produce fetal hemoglobin at levels that reduce the severity of the betaglobin disorder or its manifestations. The frequency and duration of administration will also depend upon the severity of the disorder and the general state of the patient's health, but generally will range from about 1 dose per week to about 7 doses per week, with doses of about 3 to 5 doses per week being commonly preferred. The frequency of administration may vary during a course of therapy, but generally a higher frequency is used at the beginning of treatment. The duration of treatment will typically be for the lifetime of the patient. Isobutyramide is substantially non-toxic, when administered in therapeutically effective amounts. Isobutyramide has an acute intravenous LD 50 of about 3 g per kilogram and is not mutagenic by standard assays. In life-threatening cases, it may be desirable for the treating physician to administer these compositions in substantial excesses of these amounts. In any event, the pharmaceutical compositions should provide a quantity of the isobutyramide sufficient to effectively treat the patient.

The administration of the compositions may begin at any time. The administration may begin and continue prior to birth by administration to the mother or may begin and continue after birth. The administration may also begin during the period after birth when natural production of significant amounts of gammaglobin and fetal hemoglobin takes place and continue after the patient has reached the age when natural production of gammaglobin and fetal hemoglobin has substantially ceased. In addition, the administration may commence after the so-called developmental "switch" from production of gammaglobin to betaglobin has been substantially completed and natural production of gammaglobin and fetal hemoglobin has substantially ceased. The administration may

commence during childhood or adulthood, and continue thereafter. Accordingly, the administration may begin at any age and continue throughout any ages, including beginning treatment during and after childhood.

5           The compositions and methods of this invention are also useful for research using mammals without or with a betaglobin disorder, including sheep, monkeys, other non-human primates, and transgenic mice. These compositions and methods may be used to stimulate the production of fetal hemoglobin in  
10 such mammals to obtain useful information about therapeutic treatment of these disorders.

A person skilled in the art may practice the invention based on this preceding description. The following examples are provided illustrate the invention. They are not  
15 intended to limit the scope of the invention.

#### EXAMPLE 1

##### Isobutyramide Oral Formulation

A typical pharmaceutical composition useful for oral  
20 administration of isobutyramide contains 0.7 ml sterile water, 0.3 ml sucrose syrup, and 100 mg of isobutyramide.

#### EXAMPLE 2

##### Isobutyramide Parenteral Formulation

25           A typical pharmaceutical composition for intramuscular injection contains 1 ml sterile water, and 150 mg of isobutyramide. A typical composition for intravenous infusion contains 1 ml of sterile water and 150 mg of isobutyramide.  
30

#### EXAMPLE 3

##### The Effect Of Isobutyramide On

##### Stimulating Fetal Hemoglobin In Vivo

Adult, male baboons (Papio anubis) were used as  
35 experimental animals. Three baboons were rendered anemic by daily phlebotomy for at least about 30 days, and maintained at hematocrit values of about 22% by daily phlebotomy. Each anemic baboon was treated with isobutyramide. One baboon (no.



767) received isobutyramide by oral formulation of 150 mg isobutyramide per ml of distilled sterile water administered through a naso-gastric tube three times per week for three weeks. This baboon received isobutyramide at a dosage of 600 mg per kg of body weight. Another baboon (no. 224) received isobutyramide by parenteral formulation of 150 mg isobutyramide per ml of distilled sterile water administered by intravenous infusion over a 30 minute period one time per day for three weeks. This baboon received isobutyramide at a dosage of 500 mg per kg of body weight. Another baboon (no. 207) received isobutyramide by parenteral formulation of 150 mg isobutyramide per ml of distilled sterile water administered by intravenous infusion over a 30 minute period one time per day for three days. This baboon received isobutyramide at a dosage of 500 mg per kg of body weight. The experiment was terminated with this baboon after three days due to an infection. Blood samples were taken throughout the course of treatment. Samples were assayed to determine the percentage of gammaglobin to total gamma-globin and betaglobin (Table 1) and the percentage of total reticulocytes containing gammaglobin, sometimes called "F-retics" (Table 2). The assay to measure the percentage of gammaglobin to total gamma-globin and betaglobin was by the method described in Perrine, S.P., et al., Delay In The Fetal Globin Switch In Infants Of Diabetic Mothers, New Eng.J.Med. vol.312, pp.334-338 (1985). The assay to measure the percentage of total reticulocytes containing gammaglobin was performed by the method described in Rogers, G., et al., Hematologic Response of Patients with Sick Cell Disease To Treatment with Hydroxyurea, New Eng.J.Med., vol.322, pp.1037-1045 (1990). Table 1 shows the highest percentage of gammaglobin to total gamma-globin and betaglobin achieved during treatment.

TABLE 1

Effect of Isobutyramide on Percentage of  
Gammaglobin to Total Gamma Plus Betaglobin

5	<u>Experimental Subject</u>	<u>Pre-treatment</u>	<u>Treatment</u>
	207	0	9
	224	2	11
	767	3	12

TABLE 2

Effect of Isobutyramide on Percentage  
of F-Retics to Total Reticulocytes

10	<u>Experimental Subject</u>	<u>Pre-treatment</u>	<u>Treatment</u>
	207	3	15
15	224	16	27
	767	13	17

These treatments with isobutyramide significantly stimulated the production of fetal hemoglobin as shown by the increased percentage of gammaglobin to total gamma plus betaglobin (Table 1) and the increased percentage of total reticulocytes containing gammaglobin (Table 2). The stimulation of gammaglobin production by 4-8% has been shown to produce 20-30% hemoglobin F in the blood, a level sufficient to reduce the severity of the clinical manifestations of sickle-cell anemia. Wood, W.G., et al., Haemoglobin F Synthesis in Sickle Cell Anemia: A Comparison of Saudi Arab Cases with those of African Origin, Brit.J.Hematology, vol.45, pp.431-445 (1980). This experiment shows that the administration of isobutyramide stimulates production of fetal hemoglobin to levels sufficient to reduce the severity of the clinical manifestations of betaglobin disorders. This experiment shows that such administration of isobutyramide is effective even when administration begins after the so-called developmental "switch" from production of gammaglobin to betaglobin has been substantially completed and natural production of gammaglobin and fetal hemoglobin has

substantially ceased. This experiment shows that such administration of isobutyramide is effective in adults.

#### EXAMPLE 4

##### 5                   The Effect Of Isobutyramide On                   Stimulating Fetal Hemoglobin In Vitro

Cultures of erythroid progenitors from peripheral blood of patients having a betaglobin disorder and normal subjects were cultured for about 14 days in presence of 0.2 millimoles per liter ("mM") isobutyramide and the percentages of gammaglobin to total gamma plus betaglobin were assayed substantially according to the methods described in Perrine, S.P. et al., Sodium Butyrate Enhances Fetal Globin Gene Expression in Erythroid Progenitors of Patients With Hb SS and Beta Thalassemia, Blood, vol.74, pp.454-459 (1989). The results are shown below in Table 3. The patients with sickle-cell anemia are identified as "SS" and with beta-thalassemia as "beta-thal." Ages are specified in years.

20                   TABLE 3  
                  Effect of Isobutyramide In Vitro on Percentage of  
                  Gammaglobin to Total Gamma Plus Betaglobin

	<u>Patient Disorder</u>	<u>Age</u>	<u>Pre-treatment</u>	<u>Treatment</u>
	normal	newborn	5	45
25	SS	2	17	96
	normal	newborn	20	26
	SS	10-11	22	40
	beta-thal	newborn	40	50
	SS	4	42	55
30	normal	newborn	69	72
	beta-thal	28	70	64
	SS	3	70	78
	beta-thal	22	65	98

35                   These treatments with isobutyramide significantly stimulated the production of fetal hemoglobin in culture as shown by the increased percentage of gammaglobin to total gamma-globin and betaglobin (Table 3). This experiment

confirms that the administration of isobutyramide stimulates production of fetal hemoglobin to levels sufficient to reduce the severity of the clinical manifestations of betaglobin disorders. This experiment also shows that such  
5 administration of isobutyramide is effective even when administration begins after the so-called "switch" from production of gammaglobin to betaglobin has been substantially completed and natural production of gammaglobin and fetal hemoglobin has substantially ceased. This experiment shows  
10 that such administration of isobutyramide is effective in children and adults.

#### EXAMPLE 5

##### Plasma Levels Of Isobutyramide After Administration

15 Isobutyramide was administered orally to the baboon (no.767) and parenterally to another baboon (no.224) as described in Example 3. The plasma concentrations of the isobutyramide at various times after a single oral dose to those baboons are shown in Figure 1 (measured by gas liquid  
20 chromatography and expressed in millimoles per liter ("mM")). Isobutyramide was administered orally to two human subjects. One human received a single dosage of isobutyramide orally in a formulation of 150 mg isobutyramide per ml of distilled sterile water, pH about 4.5, and at a dosage of 300 mg per kg  
25 of body weight. That human subject received separately a single dosage of isobutyramide orally in a formulation of 100 mg isobutyramide per ml of distilled sterile water, with a sucrose syrup for flavoring, and at a dosage of 150 mg per kg of body weight. Another human received a single dosage of  
30 isobutyramide orally in a formulation of 100 mg isobutyramide per ml of distilled sterile water, with a sucrose syrup for flavoring, and at a dosage of 115 mg per kg of body weight. The plasma concentrations (in mM) of the isobutyramide at various times after a single oral dose to those human subjects  
35 are shown in Figure 2. These experiments show that isobutyramide may be administered orally and parenterally to obtain plasma levels sufficient to yield therapeutic effects and with a half-life of isobutyramide sufficient for practical

administration. The half-life of isobutyramide in plasma was about 7.6 to 10.5 hours.

While I have described certain embodiments of the invention, it is apparent that the compositions and methods  
5 can be altered to provide other embodiments which utilize the compositions and methods of the invention. The scope of the invention is defined by the following claims rather than by the embodiments presented by way of example.

WHAT IS CLAIMED IS:

1. A pharmaceutical composition useful in the treatment of betaglobin disorders which comprises a therapeutically effective amount of isobutyramide and a pharmaceutically acceptable carrier.

2. A composition of claim 1 in which the carrier is suitable for oral administration.

3. A composition of claim 1 in which the isobutyramide is present in an amount sufficient to provide from about 5 to about 500 mg per kg of body weight.

4. A composition of claim 3 in which the isobutyramide is present in an amount sufficient to provide from about 10 to about 250 mg per kg of body weight.

5. A composition of claim 1 useful in the treatment of betaglobin disorders in adults.

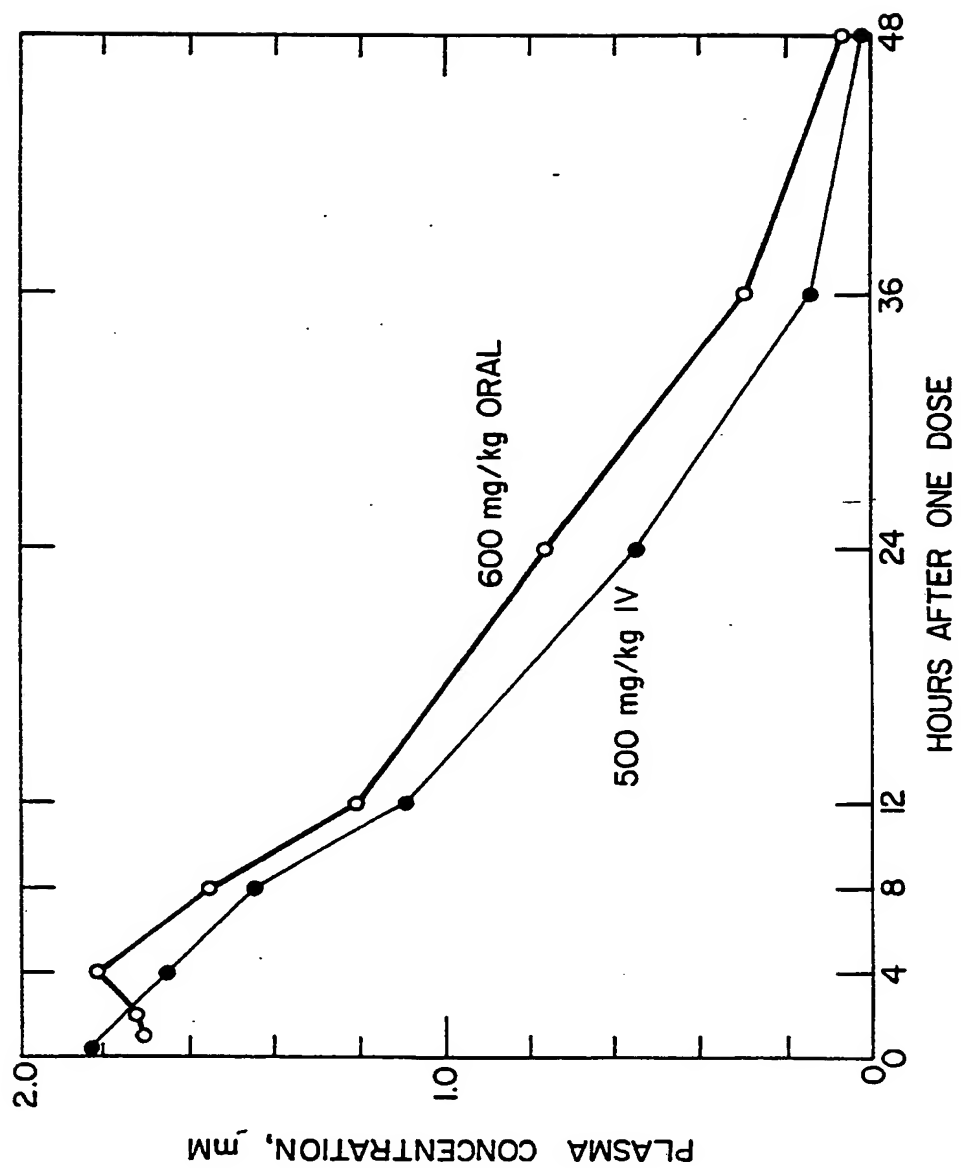


FIG. 1.

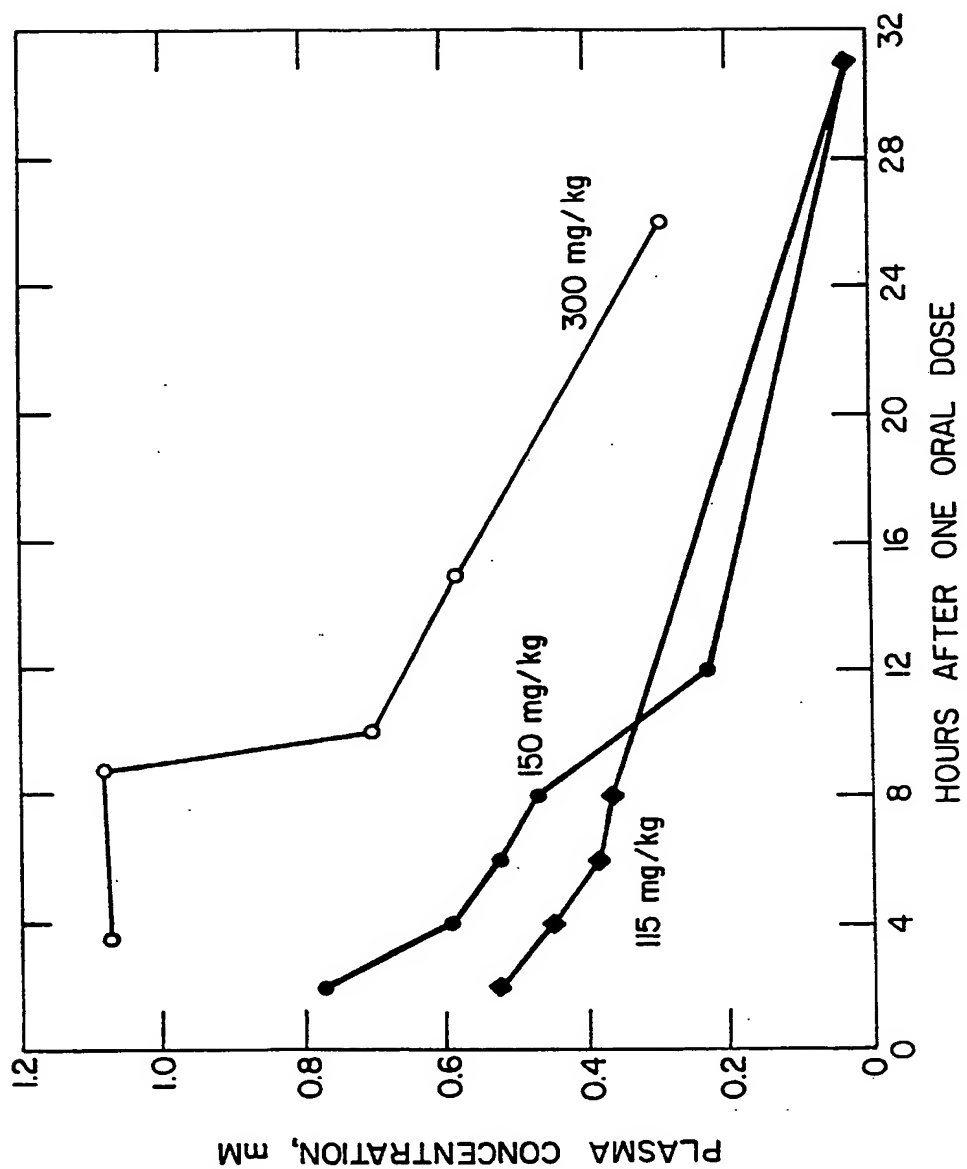


FIG. 2.



## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US93/02389

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(5) :A61K 31/16 US CL :514/629 According to International Patent Classification (IPC) or to both national classification and IPC																				
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/629  Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched  Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAS/STN AND APS ISOBUTYRAMIDE OR BUTYRIC ACID COMPOUNDS FOR BETAGLOBIA DISORDERS, ANEMIAS ETC.																				
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>																				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.																		
X	US, A, 5,025,029 (PERRINE) 18 June 1991, See columns 7 and 8.	1-5																		
Y	US, A, 4,822,821 (PERRINE) 18 April 1989, See column 2, line 55 through column 3, line 39.	1-5																		
Y	<u>The Pharmacological Basis of Therapeutics</u> (6th Edition), 1980, GOODMAN GILMAN ET AL. (Editors), See page 15.	1-5																		
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.																				
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Date of the actual completion of the international search 06 MAY 1993		Date of mailing of the international search report 01 JUN 1993																		
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## INTERNATIONAL SEARCH REPORT

International application No.  
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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Sixth Cooley's Anemia Symposium: Annals of the New York Academy of Sciences, 612:134-140, 1990, PERRINE, S. ET AL., "Stopping the Biologic Clock for globin Gene Switching", See particularly the abstract, page 135, the last full paragraph and page 136, the first full paragraph.	1-5
A	The Regulation of Hemoglobin Switching, Proceedings of the Seventh Congress on Hemoglobin Switching, 425:436, 1991, PERRINE, S. ET AL., "Pharmacologic Prevention and Reversal of Globin Gene Switching", See particularly page 425-426.	1-5